Desulfovibrio vulgaris Hildenborough responses to salt and H_2O_2 stresses

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ESPP2 Environmental Stress Pathway Project

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ABSTRACT

The response of *Desulfovibrio vulgaris* Hildenborough to salt and H_2O_2 stresses were examined by physiological, global transcriptional, metabolite, and mutagenesis analyses. The growth of *D. vulgaris* was inhibited by 250 mM NaCl or 1 mM H_2O_2. Salt adaptation (long-term NaCl exposure) increased the expression of genes involved in amino acid biosynthesis and transport, electron transfer, hydrogen oxidation, and general stress responses. Genes involved in carbon metabolism, cell motility, and phage structures were decreased in expression. Comparison of transcriptomic profiles of *D. vulgaris* responses to salt adaptation with those of salt shock (short-term NaCl exposure) showed some similarity as well as a significant difference. Metabolite assays showed that glutamate and alanine accumulated under salt adaptation, suggesting that they may be used as osmoprotectants in *D. vulgaris*. Addition of amino acids (glutamate, alanine, tryptophan) or yeast extract to the growth medium relieved salt-related growth inhibition. A conceptual model is proposed to link the observed results to currently available knowledge for further understanding the mechanisms of *D. vulgaris* adaptation to elevated NaCl.

Under H_2O_2 conditions, PerR regulon genes were significantly up-regulated, indicating the importance role of PerR in oxidative stress response. In addition, some Fur regulon genes were also strongly induced. Increased gene expression of thiol-peroxidase genes *ahpC* as well as thioredoxin reductase and thioredoxin genes indicated the involvement of thiol switch in the oxidative stress response. *rbr2* was the only significantly up-regulated H_2O_2 scavenging enzymes. The oxidative stress response of mutants Δ*perR* and Δ*fur* demonstrated that *ahpC* and *rbr2* were regulated by both Fur and PerR. The links between the up-regulated genes involved in H_2O_2 scavenging, protein fate, DNA metabolism and lipid metabolism and the down-regulated genes involved in sulfate reduction, energy production and translation were demonstrated by the gene co-expression network. The proteomics data provided further evidence at the translational level and complemented the transcriptomics data. Taken together, diverse stress resistance mechanisms may be used in *D. vulgaris* for detoxification of H_2O_2 with the up-regulation of DNA repair systems and the down-regulation of energy metabolism and protein synthesis.

MATERIALS AND METHODS

Cell culture and treatment: *D. vulgaris* cells were grown at the LS4D medium with or without salt or H2O2 treatment. Treatments were (i) 250 mM NaCl added before cell inoculation, and (ii) 1 mM H2O2 added in the mid-log phase of cell growth.

***D. vulgaris* oligonucleotide array:** 70mer oligonucleotide arrays that containing all ORFs.

Target preparation, labeling and array hybridization: Total cellular RNA was isolated and purified using TRIzol™ Reagent, and then labeled with Cy5 dye. Genomic DNA was isolated and then labeled with Cy3 dye. The labeled RNA and genomic DNA were co-hybridized to the array at 45°C with 50% formamide for 16 hrs in the dark.

Metabolite determination: Metabolite assays were conducted with capillary electrophoresis (CE) and mass spectrometric (MS) under optimal conditions.

***Construction of gene co-expression network*:** The microarray data from all six time points were used for the construction of the gene co-expression network based on the random matrix theory approach.

ACKNOWLEDGEMENTS

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D. Vulgaris Responses to H_2O_2

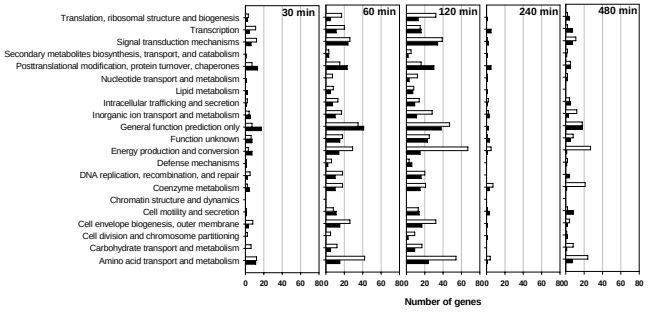

Fig. 1 Profile of the number of genes differentially expressed in the various functional categories by *D. vulgaris* Hildenborough in response to 1 mM H2O2 at different time-points after treatment (■: increase of gene expression; □: decrease of gene expression).

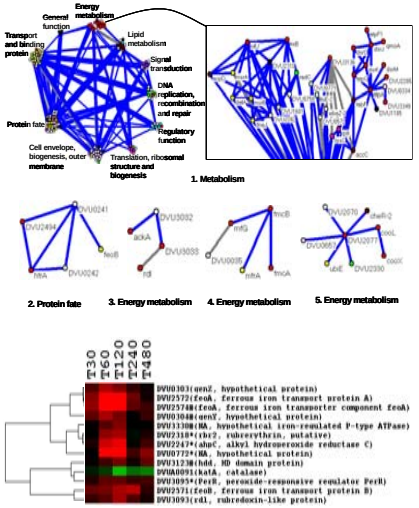

Fig. 2 Gene co-expression network for *D. vulgaris* responses to H_2O_2 stress with microarray data constructed using the random matrix theory approach. Modules with more than four genes were shown. Blue and gray indicate positive and negative correlation coefficients, respectively. In module 1, colors were assigned to nodes according to their gene function categories. In modules 2 to 5, red represents the major functional category of each module and genes in other gene categories were marked as other colors.

Fig. 3 Expression profiling of predicted genes involved in oxidative stress response, PerR regulon and Fur regulon genes.

D. Vulgaris Responses to Long-Term NaCl Exposure

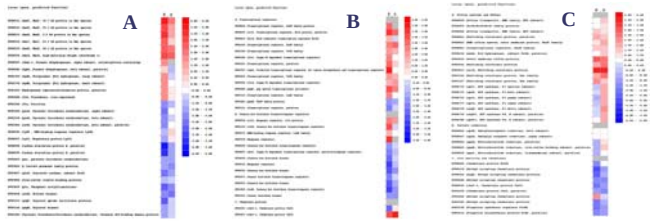

Fig. 4 The expression of genes involved in energy metabolism (A), regulatory processes (B), and efflux systems, sulfate reduction, and cell motility (C) under salt adaptation and salt shock.

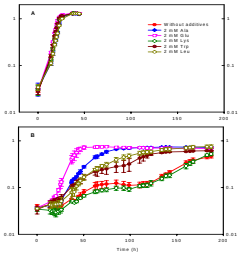

Fig. 5 Relief of salt inhibition by amino acids, Glu, Ala, Leu, Trp and Lys in the LS4D medium under the control (A) and 250 mM NaCl stress (B) conditions.

Table 1 Accumulation of amino acids in both control and NaCl treated *D. vulgaris* cells

Amino acid	Control (nMol/mg dry weight)	NaCl (nMol/mg dry weight)	P-value*	NaCl/control
Glutamate	10.25 ± 0.63	**82.82 ± 8.01**	<0.0001	**8.08**
Histidine	0.07 ± 0.00	0.20 ± 0.04	<0.0001	2.74
Serine	1.30 ± 0.12	2.54 ± 0.26	<0.0001	1.95
Betaine	1.40 ± 0.08	2.68 ± 0.58	0.0012	1.92
Lysine	0.15 ± 0.01	0.28 ± 0.06	0.0014	1.85
Glutamine	3.63 ± 0.38	**6.48 ± 0.51**	<0.0001	1.79
Alanine	9.89 ± 0.92	**17.53 ± 3.09**	0.0027	**1.77**
Leucine	0.50 ± 0.01	0.79 ± 0.05	<0.0001	1.57
Threonine	1.45 ± 0.08	2.21 ± 0.17	<0.0001	1.52
Proline	1.46 ± 0.18	2.03 ± 0.35	0.0119	1.39
Aspartate	7.41 ± 0.48	**10.21 ± 1.13**	0.0009	1.38
Glycine	8.86 ± 1.70	10.32 ± 3.16	0.3895	1.16
Isoleucine	0.69 ± 0.04	0.72 ± 0.02	0.1720	1.05
Methionine	0.05 ± 0.00	0.05 ± 0.01	1.0000	1.02
Arginine	0.94 ± 0.03	0.89 ± 0.03	0.0299	0.94
Valine	5.39 ± 1.43	4.30 ± 1.14	0.2193	0.80
Tyrosine	0.21 ± 0.00	0.13 ± 0.00	#	0.62
Phenylalanine	0.01 ± 0.00	0.01 ± 0.00	#	0.57
Tryptophan	0.005 ± 0.00	0.003 ± 0.00	#	0.55
Asparagine	ND	1.87 ± 0.41	#	

*: two-tailed p values were obtained from the Student's T test; #: a t test could not be performed since no standard deviations for those samples.

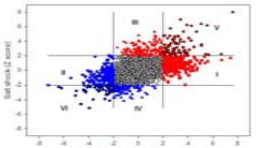

Fig. 6 A general comparison of global transcriptromic profiling of *D. vulgaris* under salt adaptation and salt shock. The Z score was set to |2.0|.

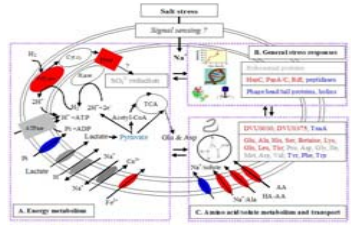

Fig. 7 A conceptual model of *D. vulgaris* responses to a long-term NaCl exposure. Color codes: red, up-regulation or increase; blue, down-regulation or decrease; gray, no significant changes.

CONCLUSIONS

- **PerR and Fur may play important roles in *D. vulgaris* responses to the oxidative stress.**
- **H_2O_2 stress of *D. vulgaris* caused the up-regulation of detoxification, protein and DNA repair systems and the down-regulation of energy metabolism and protein synthesis, and their correlations were demonstrated by the gene co-expression network.**
- **Salt adaptation increased the expression of genes involved in amino acid biosynthesis and transport, electron transfer, hydrogen oxidation, and general stress responses; genes involved in carbon metabolism, cell motility, and phage structures were decreased in expression.**
- **Comparison of transcriptomic profiles of *D. vulgaris* responses to salt adaptation with those of salt shock showed some similarity as well as difference.**
- **Glutamate and alanine accumulated under salt adaptation and also relieved salt-related growth inhibition, suggesting that they may be used as osmoprotectants in *D. vulgaris*.**